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A combination of hot air and methyl jasmonate vapor treatment alleviates chilling injury of peach fruit

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ABSTRACT

Peaches (*Prunus persica* Batsch cv Baifeng) were harvested at the firm-mature stage and treated with various combinations of methyl jasmonate (MJ) and hot air (HA). Severity of internal browning and flesh mealiness, firmness, extractable juice, total soluble solids (TSS), total acid, vitamin C and total phenolic contents were measured after 3 and 5 weeks of storage at 0 °C plus 3 d at 20 °C for shelf-life. The activities of phenylalanine ammonia-lyase (PAL, EC 4.3.1.5), superoxide dismutase (SOD, EC 1.15.1.1), polyphenol oxidase (PPO, EC 1.10.3.1), peroxidase (POD, EC 1.11.1.7), pectin-methylesterase (PME, EC 3.1.1.11) and polygalacturonase (PG, EC 3.2.1.15) were analyzed during the cold storage period. The results showed that fruit treated with 1 μmol L^{-1} MJ vapor at 38 °C for 12 h (HMJ), and heat treatment at 38 °C for 12 h and then treated with 1 μmol L^{-1} MJ vapor at 20 °C for 24 h (HA + MJ) had the highest quality and lowest percent of chilling injury symptoms. HA treatment alone significantly inhibited internal browning, but caused more severe flesh mealiness than other treatments. This side effect was counteracted by MJ. The percent of extractable juice in combined treatments was higher than that in the control, however, no significant effect was found on firmness. TSS was 23% and 25.3% higher and total acid was 59.4% and 62.5% higher in treatments of HMJ and HA + MJ, respectively, than those in control fruit after storage for 5 weeks. Vitamin C and total phenolic contents were also maintained at higher levels in combined treatments. In addition, the combined treatments resulted in higher activities of PAL, SOD and PG, and lower activities of PPO, and POD than the control. The combination of HA and MJ vapor treatment might be a useful technique to alleviate chilling injury and maintain peach fruit quality during cold storage.

1. Introduction

Peaches are very sensitive to low temperature and exhibit chilling injury after long periods of refrigeration. The main symptoms of chilling injury are internal browning and flesh mealiness that lead to a dry, grainy sand-like texture (Brummell et al., 2004; Lurie and Crisosto, 2005). Various treatments, including physical (such as modified atmosphere packaging, heat and UV-C pretreatments) and chemical methods (such as salicylic acid and methyl jasmonate (MJ)), have been applied to peach fruit to control chilling injury that occurs during low temperature storage (Fernandez-Trujillo et al., 1998a; Ebel et al., 1999; Feng et al., 2003; Girardi et al., 2005; Wang et al., 2006). However, heat treatments, including hot air (HA) treatment, hot water dipping and hot water brushing, which are more feasible for commercial application to reduce decay and prevent ripening, can also cause flesh mealiness in a number of fruit after harvest (Paull and Chen, 2000; Fallik, 2004). It has been reported that heat treatment inhibited ethylene synthesis and delayed softening in plums (Serrano et al., 2004), and when combined with controlled atmosphere storage effectively reduced internal breakdown, although flesh reddening was found in heated peaches (Malakou and Nanos, 2005; Murray et al., 2007). Therefore, it is necessary to find a combined treatment method to counteract the side effect of heat treatment on peach fruit.

MJ, as a natural plant regulator compound, plays important roles in plant growth and development, fruit ripening, and responses to environmental stress (Creelman and Mullet, 1997). In recent research, MJ has been applied to reduce postharvest diseases and chilling injury in many horticultural crops, including tomato, guava and peach fruit (Ding et al., 2002; Feng et al., 2003; González-Aguilar et al., 2004). In addition, it has been reported that MJ treatment maintained high levels of sugars and organic acids in mangoes and radishes (Wang, 1998; González-Aguilar et al.,

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2000). Thus, MJ has a potential application in postharvest treatments for alleviating chilling injury and maintaining a high-quality product.

The objective of this study was to evaluate the effect of HA combined with MJ treatment on alleviating chilling injury and maintaining quality of peach fruit. The physiological and biochemical changes associated with this treatment were also studied.

2. Materials and methods

2.1. Fruit material and treatments

Peach fruit (*Prunus persica* Batsch cv Baifeng) were hand-harvested at the firm-mature stage from a commercial orchard in Nanjing, China. The fruit were selected for uniform size, color and absence of mechanical damage, and then randomly divided into five groups of 180 fruit each. HA treatment was applied in an incubator provided with heating and humidifying systems. 1 μmol L^{-1} MJ was chosen as the optimal concentration for this experiment based on our previous research results (Feng et al., 2003; Jin et al., 2006). MJ (Aldrich, Chemical Company, Milwaukee, WI, USA) was spotted onto filter paper at final vapor concentrations of 0 (control) or 1 μmol L^{-1}, and combined HA and MJ vapor treatments were applied as follows:

(1) CK (control): fruit was incubated at 20 °C for 24 h.
(2) MJ: fruit was treated with 1 μmol L^{-1} MJ vapor in a sealed incubator at 20 °C for 24 h.
(3) HA: fruit was heat-treated in an incubator at 38 °C for 12 h.
(4) HMJ: fruit was treated with 1 μmol L^{-1} MJ vapor in the sealed incubator at 38 °C for 12 h.
(5) HA + MJ: fruit was heat-treated at 38 °C for 12 h and then treated with 1 μmol L^{-1} MJ vapor in a sealed incubator at 20 °C for 24 h.

All treatments were applied at 90–95% RH. After treatment, fruit were transferred to 0 °C for 5 weeks. Samples were collected from 5 fruit at weekly intervals for enzyme analysis. Samples were mixed and frozen immediately in liquid nitrogen, then stored at −80 °C. Another sample of 10 fruit was removed after 3 or 5 weeks of storage at 0 °C, and held at 20 °C for 3 d to simulate shelf conditions for chilling injury and quality evaluation. Each treatment was replicated three times, and the experiment was conducted twice.

2.2. Quality evaluation

Symptoms of chilling injury, including internal browning and flesh mealiness were assessed visually. The severity of internal browning or flesh mealiness was evaluated 3 d after transfer of peaches from 0 °C to 20 °C by rating on a scale of 0–4 with 0 = none, 1 = slight, 2 = moderate, 3 = moderately severe and 4 = severe. Results were expressed as internal browning or flesh mealiness index calculated using the following formula: internal browning or flesh mealiness index = $\sum$ (internal browning or flesh mealiness scale) × (number of fruit at that internal browning or flesh mealiness scale)/4 × total number of fruit in each treatment (Fernandez-Trujillo et al., 1998b).

Fruit firmness was measured on two pared sides of 10 fruit from each replicate using a TA-XT2i texture analyzer (Stable Micro System Ltd., UK) with a 5 mm diameter probe (SMSP/6). Extractable juice was determined by measuring the weight loss from 10 tissue disks (8 mm in diameter and 10 mm in thickness) after centrifuging for 10 min at 1500 × *g*. Tissue disks were supported over a wad of absorbent cotton in centrifugal tubes.

Total soluble solid (TSS) contents were determined using an Abble refractometer (14081 S/N, USA). Total acids were determined by titrating fruit juice to pH 8.1 with 0.1 mol L^{-1} NaOH, and results were expressed as g of malic acid equivalent (MAE) per 100 g of fresh weight.

Vitamin C content was measured using the method of Arakawa et al. (1981). Total phenolic content was determined according to the Folin–Ciocalteu procedure (Slinkard and Singleton, 1977). Frozen samples were homogenized in cold 80% acetone, filtered and centrifuged and results were expressed as mg of gallic acid equivalent (GAE) per 100 g of fresh weight.

2.3. Enzyme analysis

Phenylalanine ammonia-lyase (PAL) was extracted and assayed by the method described by Assis et al. (2001). Frozen tissue was homogenized in cold 0.2 mol L^{-1} sodium borate buffer at pH 8.7 containing 20 m mol L^{-1} of β-mercaptoethanol. The assay medium contained 0.1 mL of enzyme extract and 1 mL of L-phenylalanine. After incubation at 40 °C for 1 h, the reaction was stopped by adding 0.2 mL of 6 mol L^{-1} HCl. One unit of PAL activity was defined as the amount of enzyme that caused an increase in absorbance of 0.01 at 290 nm in 1 h under the specified conditions.

Superoxide dismutase (SOD) was extracted from 1 g tissue with 5 mL of 50 mmol L^{-1} sodium phosphate buffer (pH 7.8) at 4 °C. The homogenate was centrifuged at 10,000 × *g* for 20 min at 4 °C and the supernatant used to determine SOD activity by the method of Rao et al. (1996) in a final volume of 3 mL, which contained 0.1 mL crude enzyme extract. One unit of SOD activity was defined as the amount of enzyme that caused a 50% inhibition of Nitro blue tetrazolium.

For polyphenol oxidase (PPO) assay, 1 g frozen tissue was homogenized in 0.2 mol L^{-1} sodium phosphate buffer (pH 6.5) containing 1% polyvinylpyrrolidone (PVP). PPO activity was assayed following the method of Murr and Morris (1974). One unit of PPO activity was defined as the amount of enzyme that caused the increase in absorbance of 0.01 at 410 nm in 1 min under the specified conditions.

POD activity was assayed using guaiacol as a donor and H_2O_2 as a substrate according to the method of Kochba et al. (1977). One unit of POD activity was defined as an increase of 0.001 in absorbance per minute at 470 nm under the assay conditions.

Polygalacturonase (PG) activities were determined according to Zhou et al. (2000a) and one unit of activity was defined as 1 μg galacturonic acid released per mg protein per hour. Pectinmethylesterase (PME) activities were determined according to Zhou et al. (2000b) and one unit of activity was calculated as 1 mmol NaOH consumed per mg protein per hour.

2.4. Protein content

Protein content in the enzyme extracts was determined according to the Bradford (1976) method, using bovine serum albumin as a standard. Specific activity of all the enzymes was expressed as units per mg protein.

2.5. Data analysis

Experiments were performed using a completely randomized design. All statistical analyses of variance were calculated over two factors, treatment and time in storage using the SPPS statistical package (SPSS Inc., Chicago, IL, USA). The main effects were analyzed and the means were compared by Duncan's multiple range tests at a significance level of 0.05.

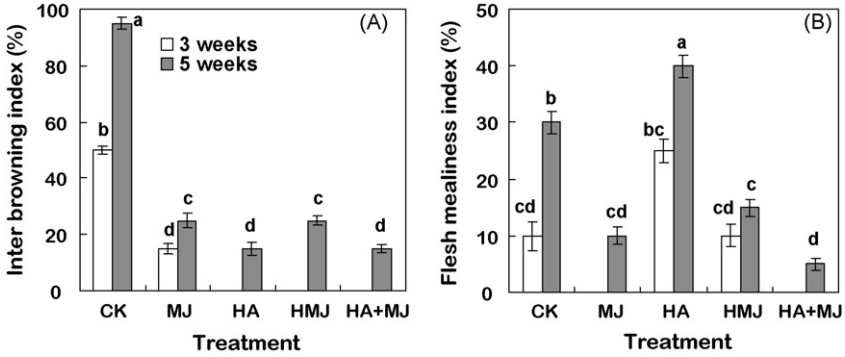

Fig. 1. Effect of methyl jasmonate (MJ), hot air (HA) and their combinations (HMJ and HA + MJ) on (A) internal browning and (B) flesh mealiness of peach fruit after 3 or 5 weeks of storage at 0 °C plus 3 d of shelf-life at 20 °C. Vertical bars represent the standard errors of the means of triplicate assays. Different letters above the bars indicate statistically significant differences at $p \leq 0.05$.

3. Results and discussion

3.1. Internal browning and flesh mealiness

Peach fruit exhibited chilling injury in the control samples after storage for 3 weeks. However, no internal browning symptoms were observed in HA, HMJ and HA + MJ treatments and no flesh mealiness symptoms were observed in MJ and HA + MJ treatments on the 3rd week. As shown in Fig. 1A, the internal browning index was 73.7%, 73.7% and 84.2% lower in the treatments of MJ, HMJ and HA + MJ, respectively, than that in control fruit after storage for 5 weeks. HA treatment alone showed the lowest internal browning index, but the flesh mealiness index was significantly higher ($p < 0.05$) than for other treatments including controls (Fig. 1B). The results indicated that MJ vapor treatment alone and combined with HA treatment could significantly decrease internal browning and flesh mealiness symptoms of peach fruit. Another adverse effect has been reported in 'Flavorcrest' peach which showed redder flesh with heated treatments than in controls (Murray et al., 2007). However, this flesh reddening symptom was not found in our experiments, which probably indicated that various peach cultivars have different responses to heat treatment and various symptoms of chilling injury. The HA + MJ treatment was more effective in inhibiting internal browning and flesh mealiness than the HMJ treatment.

Heat treatment has been found to increase the chilling tolerance of fruit and vegetable. Malakou and Nanos (2005) reported that quality of peach fruit was improved by the combination of heat treatment and modified atmosphere storage. A similar result was found in our study. Protection against chilling injury by heat treatment may relate to the accumulation of heat shock proteins (Fallik, 2004). Jasmonates are small signaling molecules in plants. Our study using a low concentration of MJ ($1\ \mu mol\ L^{-1}$) was aimed at triggering the defense mechanisms against chilling stress. After receiving signals from MJ, cells in peach fruit might activate these defense mechanisms including the production of secondary metabolites and a massive reprogramming of gene expression (Pauwels et al., 2008). Transcript levels of heat shock proteins, alternative oxidase and pathogenesis-related protein genes have been reported to increase after MJ treatments (Ding et al., 2001, 2002; Fung et al., 2004). The accumulation of these defense genes has been associated with the reduction of chilling injury in tomatoes and sweet peppers. Previous studies have also reported that MJ was effective in preventing chilling injury of avocado, grapefruit and bell pepper (Meir et al., 1996). Our data consolidated the effectiveness of MJ on alleviating chilling injury. We found that MJ treatment alone was remarkably effective in inhibiting internal browning and flesh mealiness. Furthermore, MJ could also retard flesh mealiness caused by HA treatment in peach fruit, but HA + MJ was the most effective in reducing internal browning and flesh mealiness among all treatments. The present study therefore showed that HA combined with MJ treatment was the best method to alleviate the chilling injury of peaches.

3.2. Fruit quality

There was no significant difference among all treatments on firmness after 3 or 5 weeks cold storage plus 3 d shelf-life at 20 °C (Fig. 2A). This result indicated that HA or MJ treatment did not affect the peach fruit ripening process.

MJ treatment could maintain high levels of extractable juice of peach fruit, either when treated alone or combined with HA treatment. However, the percentage of extractable juice in fruit treated only with HA was 22.6% lower than that in the control after storage for 5 weeks (Fig. 2B). Therefore, high levels of extractable juice in the MJ treatment corresponded with low levels of mealiness, whereas low levels of extractable juice in the HA treatment was associated with high percentage of mealiness.

The combination of HA and MJ treatments maintained high levels of TSS and total acids, regardless of the combined method (HMJ or HA + MJ). As shown in Fig. 2C and D, the TSS value was 23% and 25.3% higher and the percentage of total acid was 59.4% and 62.5% higher in treatments of HMJ and HA + MJ, respectively, than that in control fruit after storage for 5 weeks. In addition, MJ treatment including combination with HA maintained higher levels of vitamin C. On the contrary, HA treatment alone had little effect on this attribute. It has been found that MJ treatment can increase sugar and acid contents in guava fruit and radish (Wang, 1998; González-Aguilar et al., 2004).

All treatments increased total phenolic contents in peach fruit compared with controls. The combination of HA and MJ treatments (HMJ or HA + MJ) had better effects, but no significant difference was found between them. Similar results were reported for 'Fuji' apples, where MJ induced the accumulation of chlorogenic acid (Rudell et al., 2002).

3.3. PAL and SOD activities

As shown in Table 1, PAL and SOD activities were significantly higher ($p < 0.05$) in treated fruit than in controls in the 3rd or 5th week. The activity of PAL was significantly higher ($p < 0.05$) in the combined treatments than in the treatments alone. However, there was no significant difference in SOD activity among the treated samples.

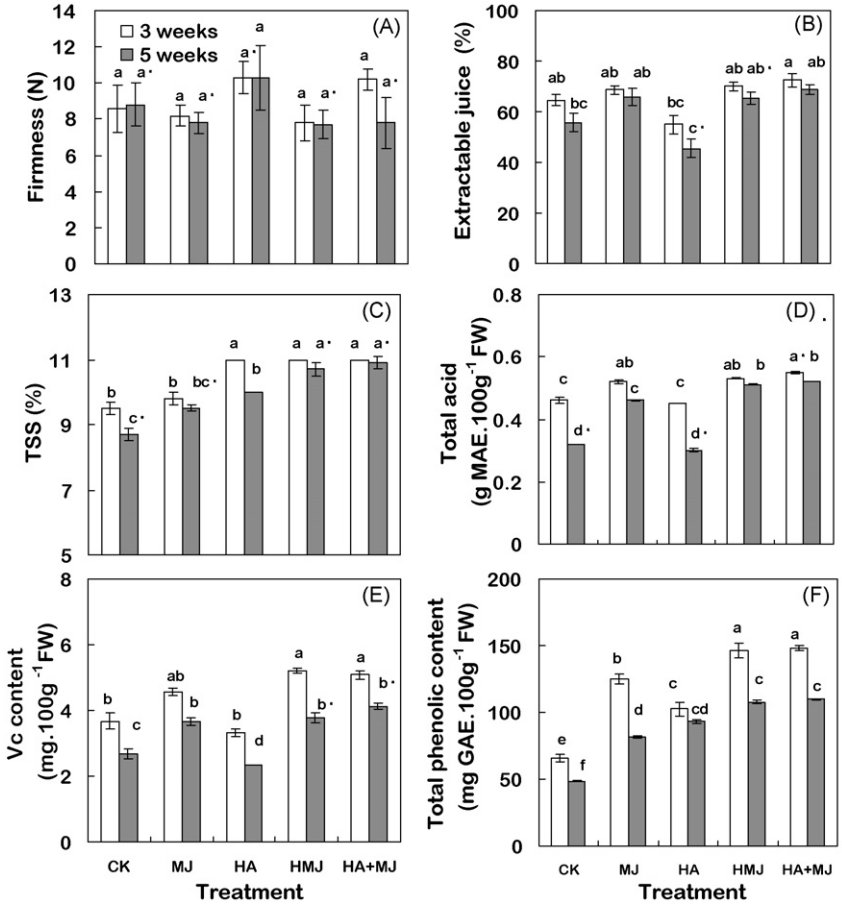

Fig. 2. Effect of methyl jasmonate (MJ), hot air (HA) and their combinations (HMJ and HA + MJ) on (A) firmness, (B) extractable juice, (C) total soluble solids, (D) total acids, (E) vitamin C and (F) total phenolic contents of peach fruit after 3 or 5 weeks of storage at 0 °C plus 3 d of shelf-life at 20 °C. Vertical bars represent the standard errors of the means of triplicate assays. Different letters above the bars indicate statistically significant differences at $p \leq 0.05$.

HA treatment, as a temperature stress, could induce a defense reaction which might protect the plant tissue from other stresses, such as cold (Fallik, 2004). MJ has been known to induce a defense response of guava fruit against chilling injury (González-Aguilar et al., 2004). In our study, PAL activity and total phenolic contents were higher in both HA and MJ treatments. This suggests that the activity of PAL and total phenolic contents might be associated with chilling tolerance in peaches.

SOD is an important antioxidant enzyme, which scavenges reactive oxygen species thereby maintaining membranes of plant tissue (Lamb and Dixon, 1997). The damage of membranes caused by cold stress is thought to be a main cause of chilling injury (Wang, 1990). Our results showed that HA combined with MJ treatment induced the activity of SOD, which would have a membrane protecting function via enhancing the antioxidant enzymes and pathogenesis-related proteins (Ding et al., 2002). Thus, the maintenance of intact

Table 1
Effect of methyl jasmonate (MJ), hot air (HA) and their combinations (HMJ and HA + MJ) on PAL, PPO, POD, SOD, PME and PG activities (U mg^{-1} protein) of peach fruit after 3 or 5 weeks of storage at 0 °C[a].

Week	Treatment	PAL	SOD	PPO	POD	PME	PG
Week 0	Initial	183.4 ± 14.8 fg	82.3 ± 11.5 d	563.2 ± 65.7 de	1198.3 ± 65.7 a	4.3 ± 0.6 e	0.16 ± 0.02 d
Week 3	Control	235.3 ± 10.4 e	102.5 ± 8.3 c	963.5 ± 81.4 b	987.4 ± 78.6 ab	7.2 ± 0.4 cd	0.16 ± 0.02 d
	MJ	352.2 ± 13.6 bc	128.5 ± 12.1 ab	687.4 ± 102.5 cd	821.5 ± 68.7 c	8.5 ± 0.2 b	0.36 ± 0.01 a
	HA	317.5 ± 14.3 cd	123.3 ± 10.5 ab	324.1 ± 116.8 e	532.4 ± 54.3 de	7.1 ± 0.2 d	0.10 ± 0.01 e
	HMJ	425.8 ± 16.3 a	124.4 ± 8.6 ab	648.5 ± 72.9 cd	685.3 ± 83.2 cd	8.4 ± 0.1 b	0.30 ± 0.02 b
	HA + MJ	396.6 ± 15.2 ab	133.6 ± 7.1 a	554.6 ± 86.3 de	587.5 ± 96.5 cd	8.8 ± 0.1 ab	0.35 ± 0.01 a
Week 5	Control	153.7 ± 22.3 g	88.4 ± 7.4 d	1436.5 ± 51.8 a	1034.5 ± 73.6 ab	8.2 ± 0.4 bc	0.06 ± 0.02 e
	MJ	295.3 ± 14.8 d	128.8 ± 8.8 ab	1024.6 ± 89.6 b	707.5 ± 63.8 cd	9.0 ± 0.1 ab	0.23 ± 0.01 c
	HA	218.2 ± 14.2 ef	123.5 ± 11.3 ab	421.5 ± 72.9 de	321.6 ± 65.6 e	8.4 ± 0.1 b	0.07 ± 0.02 e
	HMJ	300.2 ± 16.3 d	132.6 ± 12.6 a	824.5 ± 82.8 bc	658.4 ± 88.4 cd	9.8 ± 0.2 a	0.22 ± 0.01 c
	HA + MJ	305.7 ± 18.4 cd	128.8 ± 11.6 ab	623.6 ± 63.6 cd	496.3 ± 78.9 de	9.7 ± 0.4 a	0.28 ± 0.01 b

[a] Data are expressed as mean ± S.E. Different letters in the same column indicate statistically significant differences at $p \leq 0.05$.

membranes of peach tissue by HA and MJ treatments, as well as their combination, might be another mechanism of alleviating internal browning.

3.4. PPO and POD activities

PPO activity gradually increased, whereas POD activity declined after harvest in control fruit (Table 1). HA treatment significantly prevented the increase in PPO activity and enhanced the decrease in POD activity compared with controls. At the end of storage, the activities of PPO and POD in HA-treated fruit were 70.7% and 68.9%, respectively, lower than those in controls after storage for 5 weeks. MJ treatment alone and combined with HA treatments also inhibited the activities of PPO and POD; however, the effect was not as good as HA treatment alone.

Internal browning of fruit is probably related to the increase in PPO and POD activities, which could oxidize phenolic compounds to quinone or quinine-like compounds, finally appearing as polymerized brown pigments (Lill et al., 1989). Thus, the low activities of PPO and POD in HA-treated fruit were correlated with the low internal browning index. Similar results had been found in plum and fresh-cut celery treated with HA or water (Loaiza-Velarde et al., 2003; Serrano et al., 2004). MJ also had a similar function to HA treatment in inhibiting the PPO and POD activities, and the combination of MJ and HA treatments had an acceptable effect. However, the underlying factors that result in the inhibition of PPO and POD activities by HA and MJ treatments need to be further studied.

3.5. PME and PG activities

PME activity increased gradually after harvest (Table 1). MJ treatment could induce an increase in PME activity, above that of the controls, but no significant difference was found among MJ, HMJ and H + MJ treatments. In contrast, HA treatment alone was not different from the control in the 3rd or 5th week. The activity of PG in HA-treated fruit was significantly lower ($p < 0.05$) than that in controls after treatment. In contrast, MJ inhibited the decrease of PG activity, even combined with HA treatment. As shown in Table 1, PG activity was 91.7% and 133.3% higher in MJ and HA + MJ treatments, respectively, than that in controls after storage for 5 weeks.

The occurrence of flesh mealiness and woolliness in peaches or nectarines has been found to be associated with imbalance of PG and PME activities (Zhou et al., 2000b). It has been reported that flesh mealiness and woolliness are attributed to impaired solubilization of pectic substances, and the accumulation of high amounts of pectin with a low degree of esterification may cause more free water to be bound into gel, leading to less 'free juice' and causing mealiness (Zhou et al., 2000a). Our results indicated that the low activities of PME and PG, and the low PG/PME ratio in the HA treatment could lead to the development of flesh mealiness. However, PME and PG activities in HMJ and HA + MJ treatments were significantly higher ($p < 0.05$) than those in control and the HA treatment, respectively. This could be the reason that the combination of HA and MJ treatments had a low flesh mealiness index. Our results were consistent with the previous study in peaches. Even though there were differences in PME and PG activities in fruit among various treatments (Table 1), the great variations of firmness in samples within each treatment negated the effect of the treatments and rendered the differences in firmness among various treatments not significant.

4. Conclusion

In this study, we evaluated the effect of HA, MJ vapor treatment and their combination on chilling injury of peach fruit. MJ treatment alone reduced internal browning and flesh mealiness dramatically compared to the controls. The combined treatment was even more effective in alleviating chilling injury after 5 weeks of cold storage. It is possible that with optimum concentration and duration of MJ treatment alone, chilling injury symptoms could be markedly reduced. Our study showed that the HA + MJ treatment was the most effective in alleviating this disorder. Although HA treatment alone leads to severe flesh mealiness, the combined treatment presented could counteract this side effect. The combination of HA and MJ vapor treatment might be a useful technique to alleviate chilling injury and maintain fruit quality during cold storage.

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